Physiologically Based Toxicokinetic Modeling of 1,3-Butadiene Lung Metabolism in Mice Becomes More Important at Low Doses

Chris T.A. Evelo,[1] Johanna G.M. Oostendorp,[1] Wil F. ten Berge,[2] and Paul J.A. Borm[3]

[1]Department of Pharmacology, University of Limburg, Maastricht,the Netherlands;
[2]Corporate Division for Safety and Environment, DSM, Heerlen, the Netherlands;
[3]Department of Health Risk Analysis and Toxicology, University of Limburg, Maastricht, the Netherlands

This paper describes a physiologically based toxicokinetic model for 1,3-butadiene uptake, distribution, and metabolic clearance in mice. Model parameters for metabolic activity were estimated from the correspondence between computer simulation studies and experimental results as published in the literature. The parameterized model was validated with independent literature data. With the resulting model, the relative importance of lung metabolism as compared to metabolism in the liver increased with decreasing ambient air concentrations. This was due to saturation of metabolism in the alveolar area of the lung, which occurred in the simulations at ambient air concentrations well below current threshold limit values. At higher air concentration, liver metabolism became relatively more important. The tendency toward increased importance of lung metabolism at low doses indicates the necessity of careful extrapolation of *in vivo* results to low doses. Moreover, this trend may also contribute to species difference in susceptibility to the carcinogenic activity of butadiene. *Key words:* 1,3-butadiene, computer simulation, physiologically based pharmacokinetic modeling, physiologically based toxicokinetic modeling, risk evaluation, toxicokinetics. *Environ Health Perspect* 101:496–502(1993)

1,3-Butadiene (CAS no. 106–99-00) is used for the production of styrene-butadiene rubber. In 1987 more than 5 billion tons of butadiene were produced worldwide (*1*). In the Netherlands the yearly production is between 400,000 and 500,000 tons (*2*). Butadiene has been detected in cigarette smoke (*3*) and automobile exhaust (*4*) and is currently listed as one of the 189 hazardous air pollutants in the 1990 Clean Air Act Amendments (*5*). Butadiene is carcinogenic in mice (*6*). In this species, increased incidence of lung carcinoma, hemangiosarcoma of the heart, lymphoma, and bone marrow atrophy were observed (*6*). The carcinogenicity of butadiene in the rat was much less pronounced (*7,8*). Epidemiological evidence for human carcinogenicity is inconclusive, but leukemia and lymphomas are considered to be two of the largest risks (*7*). The toxicity of butadiene is attributed to its reactive metabolites 1,2-epoxybutene-3 and 1,3-diepoxybutane, which were also found to be carcinogenic in mice (*9,10*). Butadiene monoxide is formed by microsomal activity in the lung and liver of mice, rats, monkeys, and humans. There are, however, large interspecies differences in the ratio between lung and liver microsomal activity (mice > rats > humans/monkeys) (*11,12*).

These interspecies differences in kinetics interfere with conventional methods used to extrapolate data from animals to humans. Toxicokinetic models containing the relevant differences in physiological state and metabolic activity can overcome problems imposed by species differences. In recent years, toxicokinetic models have been developed rapidly (*13*). In this study we describe a model for the uptake, distribution, and metabolic degradation of butadiene in mice. The accuracy of the model was verified by simulating experimental situations described in the literature. Most of these studies were carried out with the closed-chamber technique (*14*), measuring uptake of an organic vapor by animals placed in a gas-closed chamber. After simulating exposure to various ambient air concentrations of butadiene, both the amount of butadiene present in the respective body compartments and the amount of butadiene monoxide formed in liver and lung were evaluated. The object of this study was to investigate whether the relative importance of lung and liver metabolism changes at different exposures to butadiene. Due to differences in intraorgan concentrations, lung metabolism could well be saturated at lower exposure concentrations than liver metabolism. This would have consequences for low-dose extrapolations based on *in vivo* studies performed at doses above the saturation level for alveolar metabolism. Our data demonstrate that an important shift in the relative importance of lung and liver metabolism may in fact occur at current occupational exposure levels.

Methods

Lung gas exchange, distribution, and metabolic clearance of butadiene were analyzed by a physiological toxicokinetic model (Fig. 1). Figure 2 is a schematic representation of the process of model development. The outline for the mathematical description of the model was adapted from the model for styrene described by Ramsey and Andersen (*15*). In our model, gas exchange occurs in the alveoli of the lung; metabolism occurs both in the alveolar and the bronchial areas of the lung and in the liver. Metabolic activity in the three other compartments—muscle, fat, and the vessel-rich compartment—is neglected. The bronchial, not respiratory, part of the lung was incorporated in the model to conform to the physiological blood flows. In the alveoli, two processes occur. Gas exchange can be described by the mass balance formula (See Table 1 for an explanation of the symbols used):

$$Q_{alv}C_{air} + Q_{pu}C_{v,tot} = Q_{alv}C_{exh} + Q_{pu}C_{vi,tot}. \quad (1)$$

Assuming balance between exhaled air and the blood leaving the gas exchange region:

$$C_{exh} = \frac{C_{vi,pu}}{P_{bl,air}}. \quad (2)$$

This can be combined to:

$$C_{vi,pu} = \frac{Q_{alv}C_{air} + Q_{pu}C_{v,tot}}{Q_{alv}/P_{bl,air} + Q_{pu}}. \quad (3)$$

Next, distribution over pulmonary (alveolar) tissue and metabolism therein can be described by:

$$dA_{pu}/dt = Q_{pu}\left(C_{vi,pu} - C_{v,pu}\right) - \frac{V_{max,pu} \times C_{v,pu}}{K_m + C_{v,pu}}. \quad (4)$$

The bronchial part of the lung receives arterial blood from the left side of the heart,

Address correspondence to C.T.A. Evelo, Department of Pharmacology, University of Limburg, PO Box 616, 6200 MD Maastricht, the Netherlands.
We acknowledge R. Pisters, P. van Daele, and O. Bell, who became gurus in programming an Atari ST/TT in C, and R. Zondag, R. Schins, and M. Leummens for their effort during the initial development of the model. Financial support was given by DSM Polymers BV, Geleen.
Received 28 December 1992; accepted 8 July 1993.

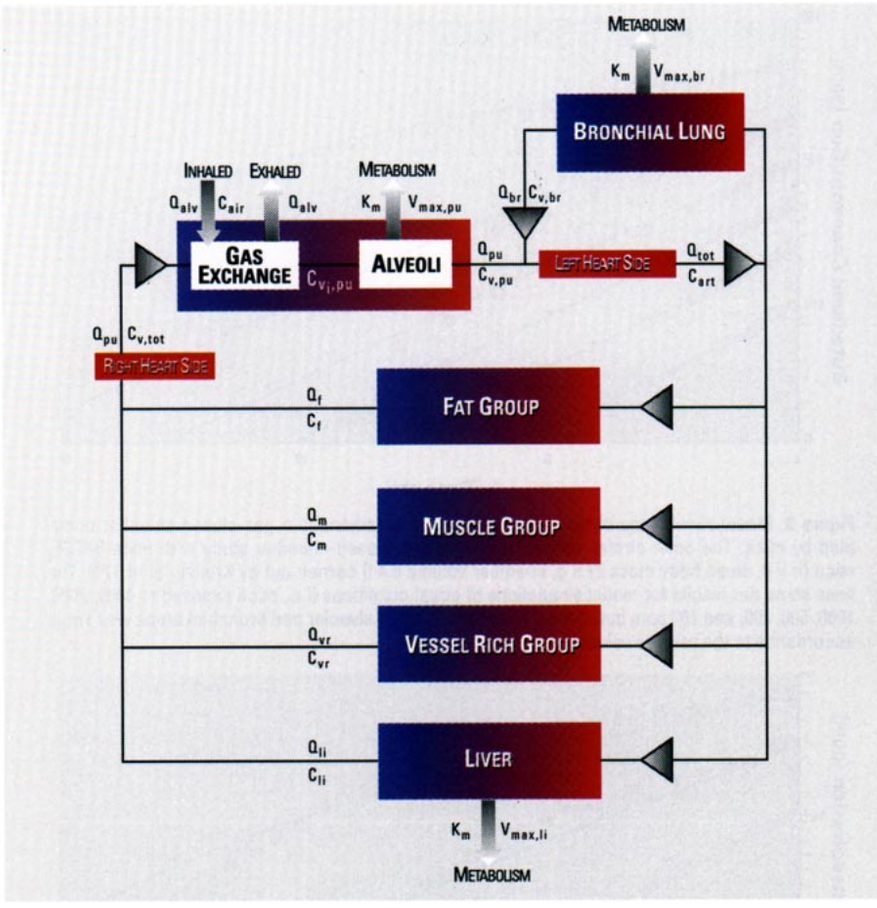

Figure 1. Physiologically based toxicokinetic model for description of butadiene distribution and metabolism in mice, rats, and humans. Gas exchange occurs in the alveoli of the lung. Metabolism occurs in both the alveolar and bronchial areas of the lung and in the liver. Metabolic activity in the three other compartments is ignored.

$$dA_{br}/dt = Q_{br}\left(C_{art} - C_{v,br}\right) - \frac{V_{max,br} \times C_{v,br}}{K_m + C_{v,br}}. \quad (5)$$

The arterial blood is a mixture of the blood returning from the alveoli and the bronchi of the lung:

$$Q_{tot}C_{art} = Q_{pu}C_{v,pu} + Q_{br}C_{v,br}, \quad (6)$$

while the summation of the two bloodstreams gives the total cardiac output of the left heart side (*16*)

$$Q_{tot} = Q_{pu} + Q_{br}. \quad (7)$$

Distribution and metabolism in the liver can be described in the same way as in the lung areas:

$$dA_{li}/dt = Q_{li}\left(C_{art} - C_{v,li}\right) - \frac{V_{max,li} \times C_{v,li}}{K_m + C_{v,li}}. \quad (8)$$

The change in the butadiene concentration in the nonactive compartments can be described by:

$$dA_x/dt = Q_x\left(C_{art} - C_{v,x}\right). \quad (9)$$

The venous concentration leaving an organ is thought to be in equilibrium with the concentration in the organ [well-stirred model (*17*)]:

$$C_{v,x} = C_x / P_{x,bl}. \quad (10)$$

Finally, the total amount of butadiene returning from the main circulation and leaving the right heart chamber toward the lung is described by:

$$Q_{pu}C_{v,tot} = Q_{li}C_{v,li} + Q_fC_{v,f} + Q_{vr}C_{v,vr} + Q_mC_{v,m}. \quad (11)$$

For validation purposes, the model was extended with the equation necessary to evaluate the changes in air concentration in a closed chamber containing n animals (*18*):

$$dC_{air}/dt = n\,Q_{alv}/V_{air}\left(C_{vi,pu}/P_{bl,air} - C_{air}\right). \quad (12)$$

The model was coded for computer simulations with our simulation package Chrisis (*19*). Parameters for simulation experiments were included in separate batch files. Simulations were executed on an Atari TT030 workstation.

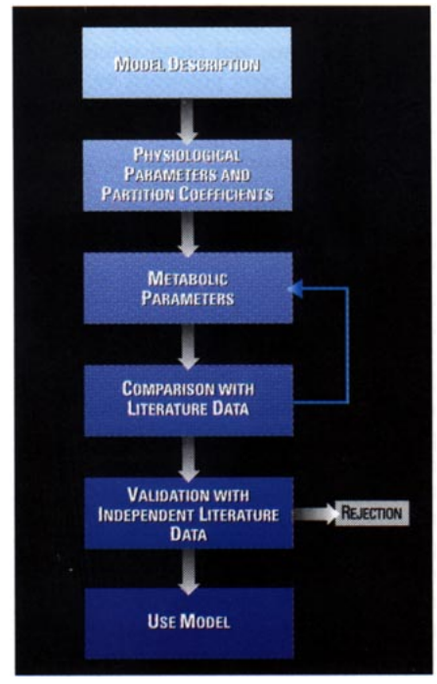

Figure 2. Schematic representation of the process of model development.

Table 1. Explanation of the symbols used

A_x	Amount of butadiene in compartment x
BMO_x	Butadiene monooxidase activity per gram tissue x
C_{air}	Butadiene concentration in inhaled air (equal to concentration in closed chamber)
C_{art}	Butadiene concentration in arterial blood (blood entering the main body compartments)
C_{exh}	Butadiene concentration in exhaled air
$C_{vi,pu}$	Intermediate butadiene concentration in the pulmonary blood after gas exchange and before distribution and metabolism
$C_{v,tot}$	Butadiene concentration in venous blood after mixing
$C_{v,x}$	Butadiene concentration in blood leaving compartment x
C_x	Butadiene concentration in compartment x
K_m	Michaelis constant for metabolic removal of butadiene
n	Number of animals used in an experiment
$P_{bl,air}$	Partition coefficient blood/air
$P_{x,bl}$	Partition coefficient between compartment x and blood
Q_{alv}	Alveolar ventilation
Q_{tot}	Cardiac output
Q_x	Blood flow through compartment x
t	time
$V_{max,x}$	Maximum metabolic rate in compartment x
V_x	Volume of compartment x
X_{br}	Value of parameter x in the bronchial part of the lung
X_f	Value of parameter x in fat tissue
X_{li}	Value of parameter x in the liver
X_{lu}	Value of parameter x in the lung
X_m	Value of parameter x in muscle tissue (lean body mass)
X_{pu}	Value of parameter x in the pulmonary (alveolar) region of the lung
X_{vr}	Value of parameter x in vessel-rich tissue

Physiological data for alveolar ventilation, blood flows, and organ volumes for mice (Table 2) were published by Travis (*20*) and were adjusted for the actual animal weight using the allometric scaling formulas as published by Fiserova (*17*). The fractional volumes and blood flows for the two separate lung compartments were taken from Greep and Weis (*16*). We estimated the tissue–blood partition coefficients and the blood–air partition coefficients used in the model by means of regression analysis of published data (*21–23*) (Table 3). We obtained the tissue–blood partition coefficients by interpolation from linear regression analysis of the tissue–blood partition coefficients on the log-transformed octanol–water partition coefficients. To obtain the blood–air partition coefficients, multiple linear regression analysis was performed on the independent variables octanol–water partition coefficient and vapor pressure after logarithmic transformation of dependent and independent variables. We calculated the ratio in maximum metabolic activity between liver and lung from the butadiene monooxidase (BMO) activity in postmitochondrial preparations of NMRI mice determined by Schmidt and Loesser (*12*), using (*24*):

$$V_{max,pu} = V_{max,li}\left(BMO_{lu}/BMO_{li} \times V_{lu}/V_{li}\right). \quad (13)$$

Recently, Csanády et al. (*11*) found that the metabolic rate of butadiene in $B6C3F_1$ mice, used in the closed-chamber study and carcinogenicity tests, is higher than the value Schmidt and Loesser reported for NMRI mice (*12*). They expressed doubt (*11*) about whether the reported values were true V_{max} values. Because we only used the ratio of the lung and liver values, this discrepancy is not likely to affect the outcome of our simulations.

The whole-body maximum metabolic activity, the affinity constant for the metabolic activity (K_m), and the most probable distribution of lung metabolic activity over the alveoli and the bronchial area were derived from model optimization with respect to the results of the closed-chamber study described by Kreiling et al. (*25*). Finally, we compared the model with the optimized values for metabolic activity to independent literature data (*26*) and used it for further simulation studies (Fig. 2).

Table 2. Physiological values for mice and rats and partition coefficients for butadiene used in the simulations and references

	Mice	Rats
Body mass (kg)	0.0275 (*25*)	0.215 (*27*)
Alveolar ventilation	24.5 (*17,20*)	118.7 (*17,20*)
Blood flows (ml/min) to:		
Liver	6.14 (*17,20*)	19.17 (*17,20*)
Fat	2.34 (*17,20*)	6.52 (*17,20*)
Muscle	3.81 (*17,20*)	11.13 (*17,20*)
Vessel-rich tissue	10.75 (*17,20*)	33.60 (*17,20*)
Bronchial lung area	1.79 (*16,17,20*)	5.514 (*16,17,20*)
Calculated from the above:		
Alveolar lung area	23.04	70.42
Cardiac output (left heart chamber)	24.83	75.93
Volumes (ml):		
Liver	1.65 (*17,20*)	8.63 (*17,20*)
Fat	2.94 (*17,20*)	14.0 (*17,20*)
Muscle	19.09 (*17,20*)	162.7 (*17,20*)
Vessel-rich tissue	1.17 (*17,20*)	9.49 (*17,20*)
Bronchial lung area	0.2 (*16,17,20*)	1.29 (*16,17,20*)
Alveolar lung area	0.18 (*16,17,20*)	1.63 (*16,17,20*)

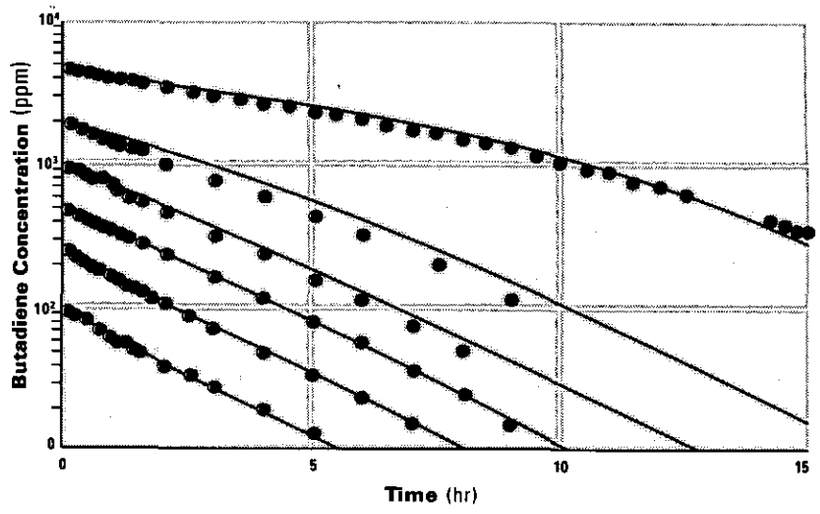

Figure 3. Model simulations of the disappearance of butadiene in a gas-closed chamber occupied by mice. The open circles show the results of a closed-chamber study with male $B6C3F_1$ mice (n = 8, mean body mass 27.5 g, chamber volume 6.4 l) carried out by Kreiling et al. (*25*). The lines show the results for model simulations of equal conditions (i.e., mice exposed to 4800, 2000, 1000, 500, 200, and 100 ppm butadiene). Metabolism in the alveolar and bronchial areas was set in accordance to the relative volumes.

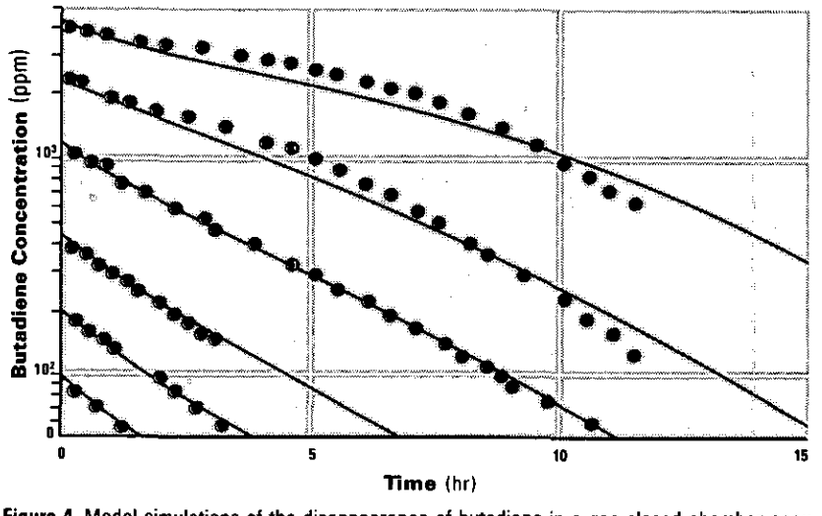

Figure 4. Model simulations of the disappearance of butadiene in a gas-closed chamber occupied by rats. The open circles show the results of a closed-chamber study with male Sprague-Dawley rats (n = 2, mean body mass 215 g, chamber volume 6.4 l) carried out by Bolt et al. (*27*). The lines show the results for model simulations of equal conditions (i.e., rats exposed to 4500, 2500, 1200, 450, 200, and 100 ppm butadiene). Metabolism in the alveolar and bronchial areas was set in accordance to the relative volumes.

Results

Determination of Metabolic Parameters

Figure 3 shows the results of simulations mimicking the closed-chamber studies with

Table 3. Partition coefficients (for both species)

Blood/air[a]	1.184, 0.603 (38)
Fat–blood	32.362
Liver–blood	2.675
Muscle–blood	1.871
Kidney–blood	1.690
Lung–blood	1.272
Brain–blood	2.355
Vessel rich–blood[b]	2.02

[a]Mean value used.
[b]Mean value of kidney–blood and brain–blood.

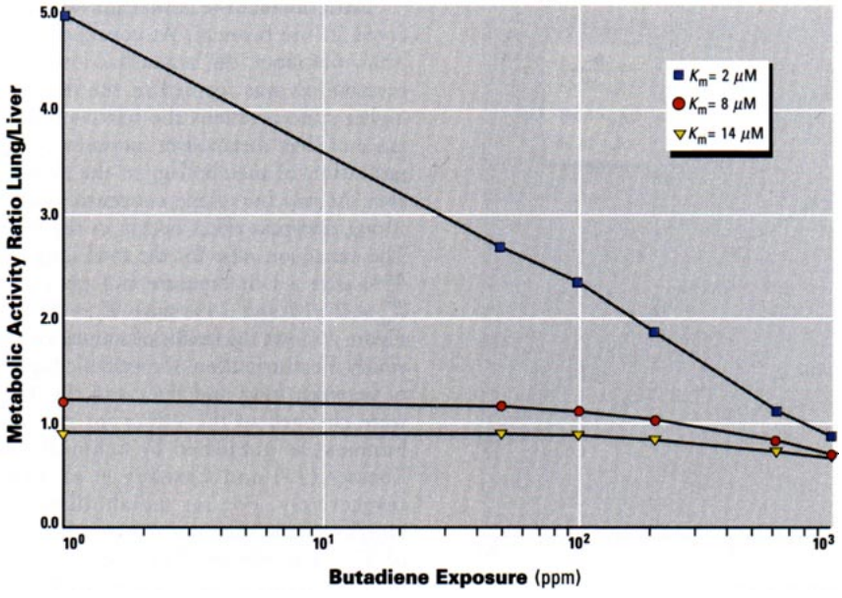

Figure 5. Ratios between total lung and liver metabolite formation in mice after an 8-hr exposure to different concentrations of butadiene. The ratios were calculated from the metabolites formed in the lung and the liver during an 8-hr exposure to 1, 50, 100, 200, 600, and 1000 ppm butadiene as calculated by model simulation.

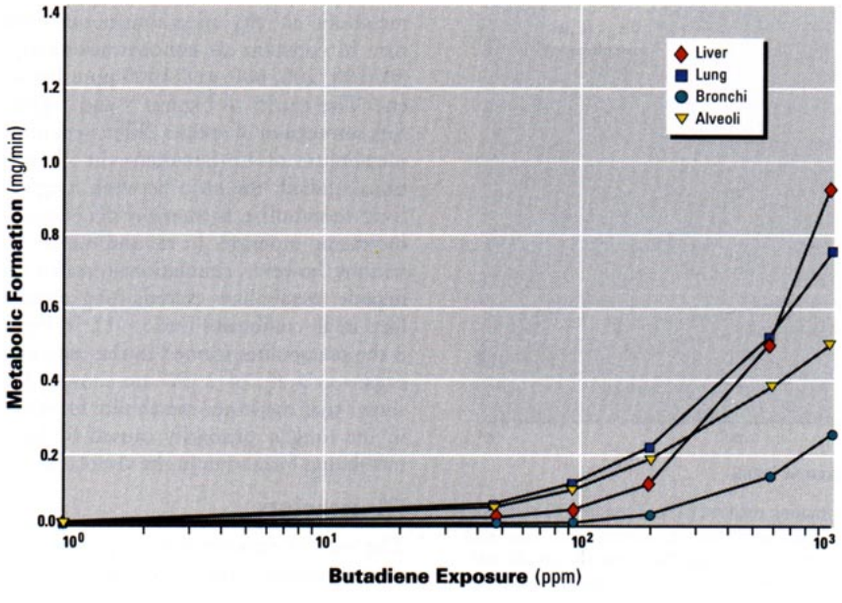

Figure 6. Metabolite formation in lung, lung parts, and liver of mice exposed to butadiene. Metabolites formed during an 8-hr exposure of mice to 1, 50, 100, 200, 600, and 1000 ppm butadiene calculated by model simulation at a K_m value of 2 μM and the ideal V_{max} of 465 μmol·hr^{-1}·kg^{-1}.

$B6C3F_1$ mice carried out by Kreiling et al. (mean body mass 27.5 g, eight animals per chamber of 6.4 l) (*25*). This best fit was obtained after optimization of model parameters, which are listed in Table 3.

The total maximal metabolic activity was determined from simulations of closed-chamber experiments with an air concentration starting at 4800 ppm. The ratio between liver and total lung metabolism was calculated as described in Methods. To find the most likely distribution of the metabolism within the lung, the following simulations were carried out: 1) all metabolism located either in the alveolar or bronchial areas, 2) an equal amount of metabolic activity in each of the two compartments, and 3) a distribution of the metabolic activity according to the relative volumes of the compartments. The best fit with the literature data was found using the last approach. We used the values of metabolic parameters found in this way for further studies.

Validation

To validate the model and its estimated parameters, we calculated the extraction ratio for butadiene and compared it to the results of a study independent from the one used for optimization of the model parameters. The calculated whole-body extraction ratio was 8.4%, whereas Dahl et al. (*26*) reported a value of 12.8%.

Rat Model

After substitution of the model with physiological (Table 2) and metabolic (Table 4) parameters appropriate for the rat, a reasonable fit with experimental closed-chamber study results for Sprague-Dawley rats, as carried out by Bolt et al. (*27*), was obtained (Fig. 4). Unfortunately, in the latter study rat weight was only given as a broad range (150–280 g). Because actual results of the simulations depend largely on animal weight, it was not possible to do a rigid test on the parameters used. Recently, Laib et al. (*28*) carried out gas-uptake studies with Sprague-Dawley rats to evaluate kinetic interactions between 1,3-butadiene, and styrene. For 1,3-butadiene, a V_{max} value was found to be 230 ± 10 μmol·hr^{-1}·kg^{-1}. This V_{max} corresponds with the V_{max} reported by Bolt et al. (220 μmol·hr^{-1}·kg^{-1}). With our model we found a best fit using a V_{max} of 200 μmol·hr^{-1}·kg^{-1}, which does not fall within the range the experimental error of Laib et al. (*28*) allows. Differences between the experimental and our simulated data were largest at high exposure. Some improvement for these curves could be obtained by using even lower V_{max} and K_m values. Finally, the rat model was validated using the study by Dahl et al. (*26*). The parameters were adjusted for the weights of the rats used, and the results were calculated. The model calculated a whole-body extraction rate of 5.2% compared to 4.3% found by Dahl et al. (26).

Lung/Liver Ratio of Metabolic Activities

Figure 5 shows how the ratio between total lung and liver metabolic activity in mice depends on the ambient air concentration. The data are the model calculated ratios for metabolites formed after 8 hr when

Table 4. Metabolic parameters for mice and rats found by optimization of the model toward literature data

	Mice	Rats
$V_{max,tot}$ (μmol·hr^{-1}·kg^{-1})	465	200
$V_{max,li}$ (μmol·hr^{-1}·kg^{-1})	318	171
$V_{max,br}$ (μmol·hr^{-1}·kg^{-1})	77	13
$V_{max,pu}$ (μmol·hr^{-1}·kg^{-1})	70	16
K_m (μM)	8	5

Where $V_{max,x}$ is the maximum rate of metabolic butadiene removal for tissue *x*; tot, li, br, and pu stand for total, liver, bronchial, and pulmonary (alveolar), respectively. K_m is the Michaelis constant.

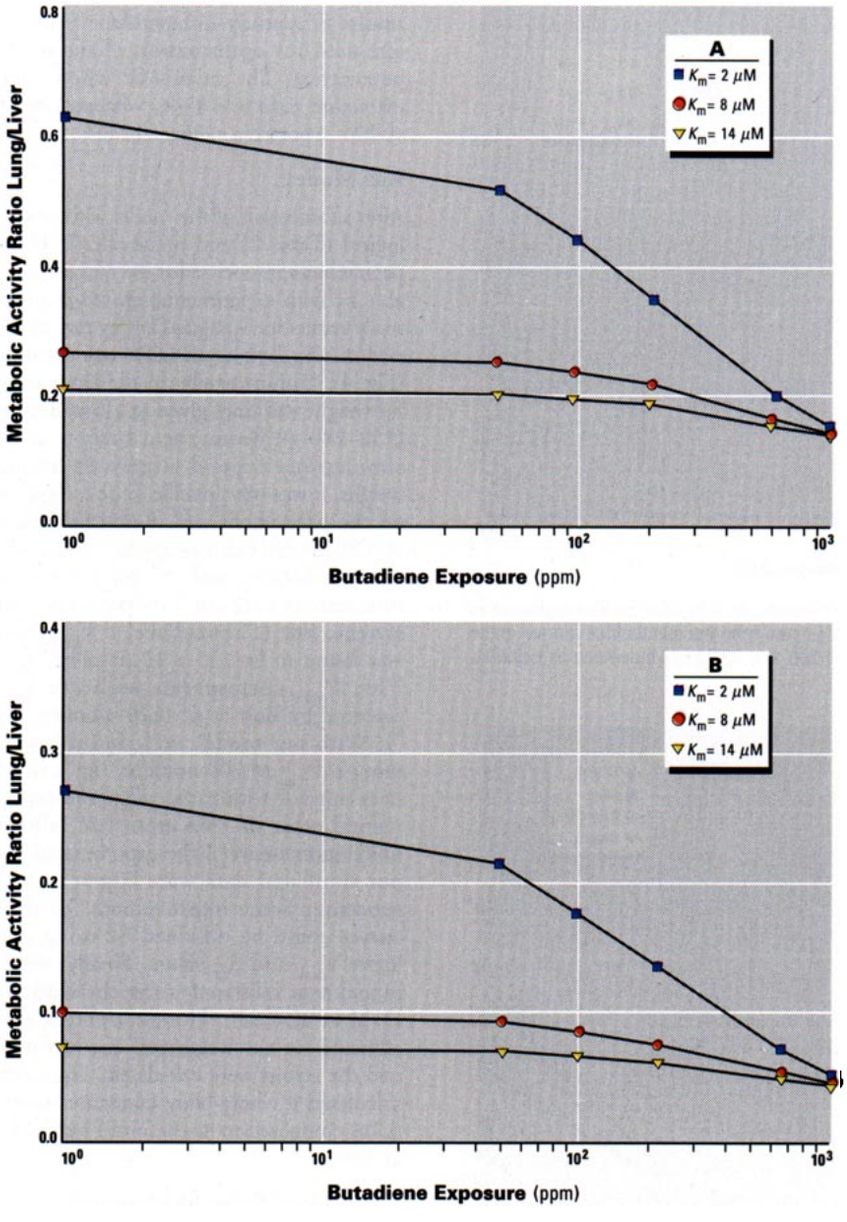

Figure 7. Ratios between total lung and liver metabolite formation with rat (A) and human (B) metabolic conditions after exposure to butadiene. To examine the species differences among mice, rats, and humans, the same physiological parameters were used as for mice except that species-specific values were taken for the ratio between lung–liver metabolism and V_{max}. For rat metabolism the lung-liver ratio used was 0.5 and the value of V_{max} was 200 μmol·hr^{-1}·kg^{-1}. For human metabolism these values were 0.15 and 237 μmol·hr^{-1}·kg^{-1}, respectively. Simulation results were calculated for the ratios between total lung and liver metabolic activity after continuous exposure to constant air concentrations of 1, 50, 100, 200, 600, and 1000 ppm butadiene.

mice were exposed continuously to 1, 50, 100, 200, 600, and 1000 ppm butadiene. Sensitivity analyses revealed that fitting the model to the experimental closed-chamber study is relatively insensitive to errors in the K_m value. A reasonable fit could still be obtained at K_m values 6 μM higher and lower than the optimal K_m value. Therefore, the ratios for lung and liver metabolism were calculated not only for the ideal K_m of 8 μM but also for 2 and 14 μM. In all cases the ratio between lung and liver metabolism increased when the exposure concentrations decreased below 1000 ppm. This increase was most pronounced in simulations using the lowest K_m values, but for the K_m value of 8 μM there was still a 40% increase between the values calculated at 1000 ppm and those calculated at 1 ppm. Figure 6 shows the rate of metabolite formation under these conditions in the separate compartments: liver, total lung, and the bronchial and alveolar areas. As Figure 6 is meant to clarify the phenomenon shown in Figure 5, the low K_m value of 2 μM is used.

Little metabolite formation was observed in the bronchi. At concentrations below 600 ppm, the largest fraction of the metabolites was formed in the lung. At higher concentrations the relative importance of liver metabolism increases due to saturation of metabolism in the alveolar area. At low butadiene concentrations, a strong first-pass effect occurs in the lung. The extraction ratio for the total lung was 45% after a 1-hr exposure at 1 ppm with K_m = 2 μM and 14% with K_m = 8 μM. Figure 7 shows the results of simulations in which the distribution of metabolic capacity between lung and liver and the V_{max} were changed to the values for rats and humans as published by Schmidt and Loesser (*12*) and Csanády et al. (*11*), respectively. For rat metabolism, the lung/liver ratio used was 0.5 and the value of V_{max} was 200 μmol·hr^{-1}·kg^{-1}. For human metabolism, these values were 0.15 and 237 μmol·hr^{-1}·kg^{-1}, respectively. The simulations were executed with the ideal K_m of 8 μM for mice as well as for 2 and 14 μM. Simulation results were calculated for the ratios between total lung and liver metabolic activity after continuous exposure to constant air concentrations of 1, 50, 100, 200, 600, and 1000 ppm butadiene. The results in Figures 5 and 7 give a first impression of species differences in the metabolism of 1,3-butadiene. In all situations studied, the ratio between lung and liver metabolite formation decreased at increasing exposure. In rat and human situations, however, simulations revealed that hepatic metabolism exceeds lung metabolism in all conditions (ratio < 1). In Figure 8 the metabolites formed in the individual organs at a K_m of 2 μM are shown. This shows that the lower metabolite formation in the lung is primarily caused by lower metabolite formation in the alveolar area.

Discussion

The model described here predicts the uptake, distribution, and metabolic clearance of inhaled butadiene in mice. To obtain a good agreement between the literature data and simulation output, it was necessary to take into account the bifurcated blood supply of the lungs. After optimization of the model parameters for metabolic activity, the results obtained by computer simulation were in good agreement with the experimental data published by Kreiling et al. (*25*). The extraction ratio found by Dahl et al. (*26*) was about 30% lower than the value calculated from our simulations. This difference can be well accounted for by differences in animals and experimental conditions between the studies used for fitting and validation. Bond et al. (*29*) found depressed butadiene metabolism in lung microsomes from mice

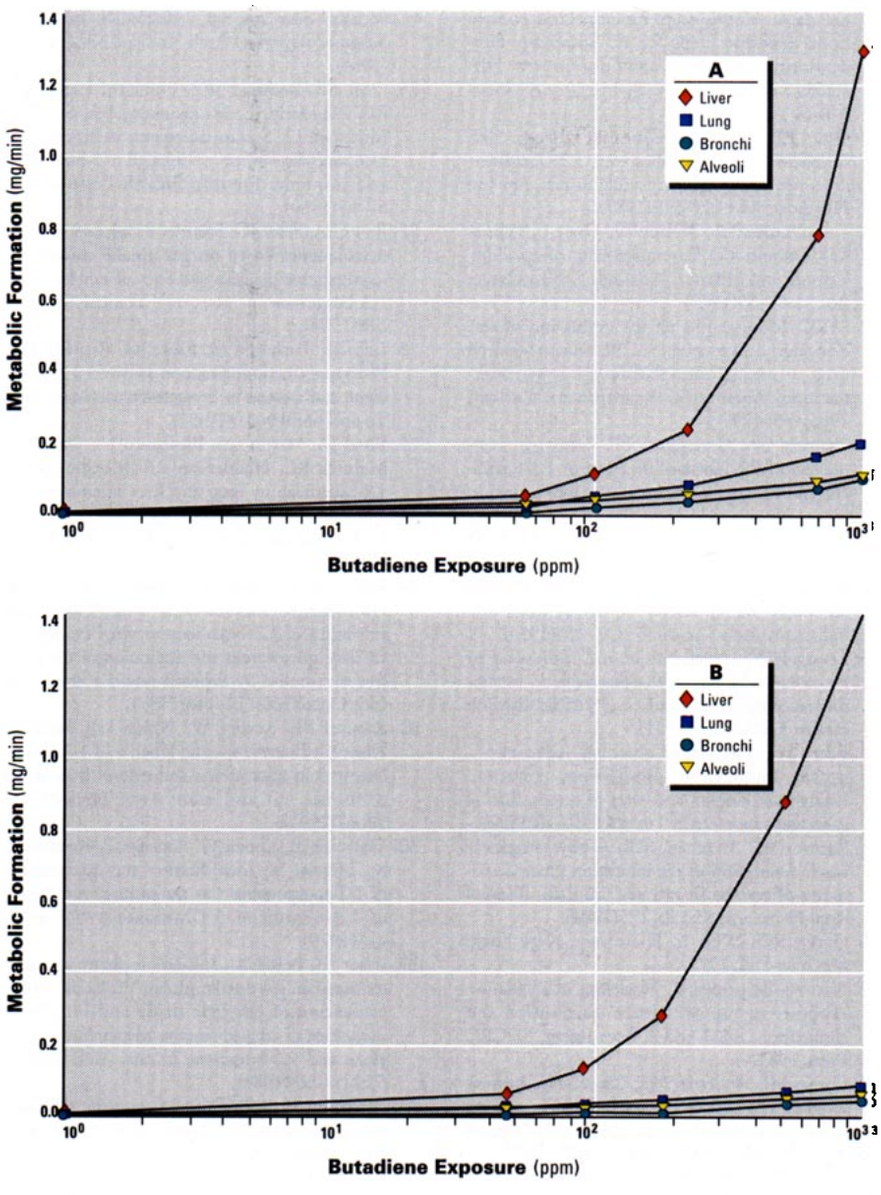

Figure 8. Metabolite formation in lung, lung parts, and liver with rat (A) and human (B) metabolic conditions after exposure to butadiene. Simulation results shown using rat- and human-specific values for V_{max} and the ratio between lung and liver metabolism at a K_m of 2 µM. Conditions were the same as in Figure 7.

and rats repeatedly exposed (5 days, 6 hr/day) to high concentrations (740 and 7600 ppm, respectively) of butadiene. If this phenomenon did occur during the closed-chamber studies, overestimation of butadiene removal after longer exposures at high concentrations is expected. Although this is consistent with the fact that the calculated line for butadiene left in the chamber in the experiment starting at 4800 ppm (Fig. 3) bends below the experimental values at exposure times exceeding 12 hr, the effect seems to be of minor importance for the exposure times used.

After substitution of relevant rat parameters (Table 2) into our model, simulations were in reasonable agreement with experimental data in rats from a closed-chamber study published by Bolt et al. (*27*). At higher concentrations, deviations between experimental data and simulation results still exist. An important reason for these deviations is the relatively fast disappearance of butadiene after longer exposure times (>6 hr) at high starting concentrations (2500 ppm and higher) in the closed-chamber study. In fact, the disappearance at equal remaining chamber concentrations is faster than what was found for studies starting at lower concentrations. This indicates that some kind of induction of metabolism may have occurred. (Note that this effect is opposite to the depression after prolonged exposure described above.) The description of the experimental conditions in the literature was inadequate for rigid control of the simulation results. For this reason, no further simulations were done with the rat model, although calculated whole-body extraction ratios were in good agreement with those reported by Dahl et al.

Butadiene is metabolized both *in vitro* by rat, mouse, and monkey liver and lung postmitochondrial preparations (*11,12, 29–32*) and in these animals *in vivo* (*26,33*) to 1,2-epoxy-butene-3 (butadiene monoxide, vinyl oxirane). This reactive epoxide can be hydrolyzed to 3-butene-1,2-diol by epoxide hydrolase; it can also be oxidized further to di-epoxy-butane (*34*) by cytochrome P450-dependent activity, and it can be conjugated with glutathione (*35*). Kreuzer et al. (*31*) detected no NADPH-dependent metabolism of butadiene monoxide in microsomes from mice, rats and humans. Csanády et al. (*11*) found that formation of diepoxybutane from butadiene monoxide could be quantified only in mouse liver microsomal systems. They also reported that enzymatic hydrolysis is highest in human liver tissue compared to mice and rats, whereas capacity for glutathione conjugation is highest in mice. Furthermore, Csanády et al. found butadiene monoxide formation in human lung (n =12) microsomes, in contrast to Schmidt and Loesser (*12*), who did not detect any butadiene monoxide in analogous preparations of one subject. Recently, Dahl et al. (*36*) reported that the concentrations of total 1,3-butadiene metabolites in the blood of monkeys were 5–50 times lower than in mouse and 4–14 times lower than in rats. Therefore, species differences in the activity and localization of the formation of this activated metabolite might be important for risk evaluation.

The results of our simulations show that in mice the relative importance of metabolite formation in the lung is higher than would be expected solely from the distribution of the metabolic activity. This is especially true at lower (< 200 ppm) exposures. The cause for this phenomenon lies in the strong first-pass effect in the lung, which results in much higher concentrations in alveolar tissue than in all other tissues. At 1 ppm exposure and with a K_m of 2 µM, the concentration in alveolar tissue was six times higher than in liver tissue, and for a K_m of 8 µM it was still two times higher. In addition to this first-pass effect, the high blood flow to the alveolar area also contributes to the importance of lung metabolism. When the distribution between lung and liver metabolism was changed to the values published for rats and humans, a shift to higher relative importance of lung metabolism at low doses was also found. However, although the shift in mice made the lung the most important metabolic organ at low exposures, this was not so in the rat and human-like situations. In mice the lung/liver ratio

shifted from 0.67 to 1.19 at a K_m of 8 μM and from 0.82 to 4.89 at a K_m of 2 μM. In simulations using human parameters for metabolism, these shifts were from 0.047 to 0.102 and from 0.053 to 0.27, respectively. The relatively high rate of activation found in the lungs of mice might be responsible for the appearance of lung carcinoma and could also contribute to the formation of heart hemangiosarcoma. Therefore, our findings indicate that the high sensitivity of mice is not only due to species-dependent differences in metabolic activity, but also to resulting shifts in the relative importance of organ-specific metabolism.

The original carcinogenicity studies in $B6C3F_1$ mice were carried out at butadiene concentrations of 625 and 1250 ppm (*5*). In more recent studies using much lower concentrations, increased incidences of alveolar–bronchiolar neoplasms in female mice were found after 2-year exposures as low as 6.25 ppm (*37*). This high sensitivity to alveolar–bronchiolar neoplasm formation at low doses, as found in mice, can be explained by the relatively high rate of lung metabolism at low exposure levels indicated by our simulation studies. In fact, two phenomena occur: 1) lung metabolism at low exposures is higher than would be expected from linear extrapolation from data obtained at high exposures, and 2) the relative importance of metabolite formation in the lung as compared to that in the liver is higher than would be expected from findings at high doses. This could result in a shift of the relative importance of liver neoplasms to lung neoplasms at lower doses.

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